Berg Report Exhibit D

United States Patent [19]

Roth

[11] **Patent Number: 4,681,893**

[45] **Date of Patent: Jul. 21, 1987**

[54] **TRANS-6-[2-(3- OR 4-CARBOXAMIDO-SUBSTITUTED PYRROL-1-YL)ALKYL]-4-HYDROXYPYRAN-2-ONE INHIBITORS OF CHOLESTEROL SYNTHESIS**

[75] Inventor: **Bruce D. Roth**, Ann Arbor, Mich.

[73] Assignee: **Warner-Lambert Company**, Morris Plains, N.J.

[21] Appl. No.: **868,867**

[22] Filed: **May 30, 1986**

[51] **Int. Cl.**[4] **A61K 31/40**; A61K 31/35; C07D 207/327

[52] **U.S. Cl.** **514/422**; 514/423; 546/256; 546/275; 548/517; 548/537

[58] **Field of Search** 548/517, 537; 514/422, 514/423

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,983,140	9/1976	Endo et al.	549/292
4,049,495	9/1977	Endo et al.	435/125
4,137,322	1/1979	Endo et al.	548/344 X
4,198,425	4/1980	Mitsui et al.	514/460
4,255,444	3/1981	Oka et al.	549/292 X
4,262,013	4/1981	Mitsui et al.	549/292 X
4,375,475	3/1983	Willard et al.	514/460

OTHER PUBLICATIONS

Singer, et al.; Proc. Soc. Exper. Biol. Med.; vol. 102, pp. 370–373, (1959).

Hulcher; Arch. Biochem. Biophys., vol. 146, pp. 422–427, (1971).

Brown, et al.; New England Jour. of Med., vol. 305, No. 9, pp. 515–517, (1981).

Brown, et al.; J. Chem. Soc. Perkin I, (1976), pp. 1165–1170.

Journal of the Americas Medical Assoc.; (1984), vol. 251, pp. 351–364, 365–374.

Primary Examiner—Joseph Paul Brust

Attorney, Agent, or Firm—Jerry F. Janssen

[57] **ABSTRACT**

Certain trans-6-[2-(3- or 4-carboxamido-substituted pyrrol-1-yl)alkyl]-4-hydroxypyran-2-ones and the corresponding ring-opened acids derived therefrom which are potent inhibitors of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG CoA reductase and are thus useful hypolipidemic or hypocholesterolemic agents. Pharmaceutical compositions containing such compounds, and a method of inhibiting the biosynthesis of cholesterol employing such pharmaceutical compositions are also disclosed.

9 Claims, No Drawings

TRANS-6-[2-(3- OR 4-CARBOXAMIDO-SUBSTITUTED PYRROL-1-YL)ALKYL]-4-HYDROXYPYRAN-2-ONE INHIBITORS OF CHOLESTEROL SYNTHESIS

BACKGROUND OF THE INVENTION

The present invention is related to compounds and pharmaceutical compositions useful as hypocholesterolemic and hypolipidemic agents. More particularly, this invention concerns certain trans-6-[2-(3- or 4-carboxamidosubstitutedpyrrol-1-yl)alkyl]-4-hydroxypyran-2-ones and the corresponding ring-opened acids derived therefrom which are potent inhibitors of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG CoA reductase), pharmaceutical compositions containing such compounds, and a method of inhibiting the biosynthesis of cholesterol employing such pharmaceutical compositions.

High levels of blood cholesterol and blood lipids are conditions involved in the onset of arteriosclerosis. It is well known that inhibitors of HMG-CoA reductase are effective in lowering the level of blood plasma cholesterol, especially low density lipoprotein cholesterol (LDL-C), in man (cf. M. S. Brown and J. L. Goldstein, *New England Journal of Medicine*, 305, No. 9, 515–517 (1981). It has now been established that lowering LDL-C levels affords protection from coronary heart disease (cf. *Journal of the American Medical Association*, 251, No. 3, 351–374 (1984).

Moreover, it is known that certain derivatives of mevalonic acid (3,5-dihydroxy-3-methylpentanoic acid) and the corresponding ring-closed lactone form, mevalonolactone, inhibit the biosynthesis of cholesterol (cf. F. M. Singer et al., *Proc. Soc. Exper. Biol. Med.*, 102: 370 (1959) and F. H. Hulcher, *Arch. Biochem. Biophys.*, 146: 422 (1971)).

U.S. Pat. Nos. 3,983,140; 4,049,495 and 4,137,322 disclose the fermentative production of a natural product, now called compactin, having an inhibitory effect on cholesterol biosynthesis. Compactin has been shown to have a complex structure which includes a mevalonolactone moiety (Brown et al., *J. Chem. Soc. Perkin* I (1976) 1165.

U.S. Pat. No. 4,255,444 to Oka et al. discloses several synthetic derivatives of mevalonolactone having antilipidemic activity.

U.S. Pat. Nos. 4,198,425 and 4,262,013 to Mitsue et al. disclose aralkyl derivatives of mevalonolactone which are useful in the treatment of hyperlipidemia.

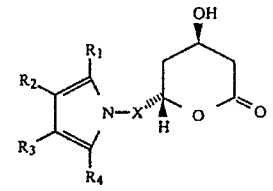
U.S. Pat. no. 4,375,475 to Willard et al. discloses certain substituted 4-hydroxytetrahydropyran-2-ones which, in the 4(R)-trans-stereoisomeric form, are inhibitors of cholesterol biosynthesis.

Published PCT application No. WO 84/01231 discloses certain indole analogs and derivatives of mevalonolactone having utility as hypolipoproteinemic and antiatherosclerotic agents.

SUMMARY OF THE INVENTION

In accordance with the present invention, there are provided certain trans-6-[2-(3- or 4-carboxamido-substituted pyrrol-1-yl)alkyl]-4-hydroxypyran-2-ones and the corresponding ring-opened hydroxy-acids derived therefrom which are potent inhibitors of cholesterol biosynthesis by virtue of their ability to inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase).

In particular, in its broadest aspect the present invention provides compounds of structural formula I



I

wherein X is $-CH_2-$, $-CH_2CH_2-$, $-CH_2CH_2CH_2-$ or $-CH_2CH(CH_3)-$.

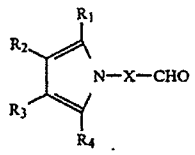
R_1 is 1-naphthyl; 2-naphthyl; cyclohexyl; norbornenyl; 2-, 3-, or 4-pyridinyl; phenyl, phenyl substituted with fluorine, chlorine, bromine, hydroxyl; trifluoromethyl; alkyl of from one to four carbon atoms, alkoxy of from one to four carbon atoms, or alkanoyloxy of from two to eight carbon atoms.

Either R_2 or R_3 is $-CONR_5R_6$ where R_5 and R_6 are independently hydrogen; alkyl of from one to six carbon atoms; 2-, 3-, or 4-pyridinyl; phenyl; phenyl substituted with fluorine, chlorine, bromine, cyano, trifluoromethyl, or carboalkoxy of from three to eight carbon atoms; and the other of R_2 or R_3 is hydrogen; alkyl of from one to six carbon atoms; cyclopropyl; cyclobutyl; cyclopentyl; cyclohexyl; phenyl; or phenyl substituted with fluorine, chlorine, bromine, hydroxyl; trifluoromethyl; alkyl of from one to four carbon atoms, alkoxy of from one to four carbon atoms, or alkanoyloxy of from two to eight carbon atoms.

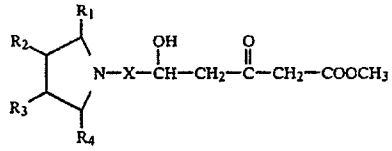
R_4 is alkyl of from one to six carbon atoms; cyclopropyl; cyclobutyl; cyclopentyl; cyclohexyl; or trifluoromethyl.

Also contemplated as falling within the scope of the present invention are the hydroxy acids, and pharmaceutically acceptable salts thereof, derived from the opening of the lactone ring of the compounds of structural formula I above.

In another aspect of the present invention, there is provided a method of preparing the compounds of structural formula I above which comprises the steps of (a) first reacting a substituted [(pyrrol-1-yl)alkyl]aldehyde compound of the formula

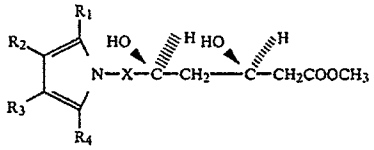


with the dilithio or sodio-lithio salt of methyl acetoacetate to form a compound of the structure



(b) reducing the product of step (a) with a trialkylborane compound such as tributylborane in the presence of sodium borohydride in an inert solvent;

(c) oxidizing the product of step (b) with alkaline aqueous hydrogen peroxide solution to produce a compound of the formula



and

(d) cyclizing the product step (c) to a lactone of formula I above by heating in an inert solvent such as toluene or, alternatively converting the product of step (c) to a pharmaceutically acceptable salt by conventional methods.

In yet another aspect, the present invention provides pharmaceutical compositions useful as hypolipidemic or hypocholesterolemic agents comprising a hypolipidemic or hypocholesterolemic effective amount of a compound in accordance with this invention as set forth above, in combination with a pharmaceutically acceptable carrier.

In another aspect, the present invention provides a method of inhibiting cholesterol biosynthesis in a patient in need of such treatment by administering an effective amount of a pharmaceutical composition as defined above.

DETAILED DESCRIPTION

The compounds of the present invention comprise a class of trans-6-[2-(3- or 4-carboxamidosubstituted pyrrol-1-yl)alkyl]-4-hydroxypyran-2-ones in which the pyran-2-one moiety is attached, through an alkyl chain, to the substituted pyrrole nucleus at the nitrogen, or 1-position, of the pyrrole. The alkyl group may be methylene, ethylene, propylene, or methylethylene. The preferred alkyl chain linking the substituted pyrrole nucleus and the 4-hydroxypyran-2-one ring is ethylene.

The compounds of structural formula I above possess two asymmetric carbon centers, one at the 4-hydroxy position of the pyran-2-one ring, and the other at the 6-position of the pyran-2-one ring where the alkylpyrrole group is attached. This asymmetry gives rise to four possible isomers, two of which are the R-cis- and S-cis-isomers and the other two of which are the R-trans- and S-trans-isomers. This invention contemplates only the trans- form of the compounds of formula I above.

In the compounds of the present invention, position 2 of the substituted pyrrole nucleus is substituted with 1-naphthyl; 2-naphthyl; cyclohexyl; norbornenyl; 2-, 3-, or 4-pyridinyl; phenyl, phenyl substituted with fluorine, chlorine, bromine, hydroxyl; trifluoromethyl; alkyl of from one to four carbon atoms, alkoxy of from one to four carbon atoms, or alkanoyloxy of from two to eight carbon atoms. Preferred substituent groups at the 2-position of the pyrrole nucleus are phenyl and substituted phenyl.

In the compounds of this invention, position 5 of the pyrrole nucleus is substituted with alkyl of from one to six carbon atoms; cyclopropyl; cyclobutyl; cyclopentyl; cyclohexyl; or trifluoromethyl. Preferred substituents are alkyl or trifluoromethyl with isopropyl being particularly preferred.

The preferred reaction sequence which is used to prepare compounds of the present invention involves the cycloaddition of a disubstituted acetylene, in which one substituent is carboxamido or N-substituted carboxamido, to an appropriately substituted N-acylaminocarboxylic acid to form a substituted pyrrole. This addition may occur in either of two ways, leading to a substituted pyrrole addition product in which the carboxamido substituent resides on either carbon 3 or 4 of the pyrrole nucleus.

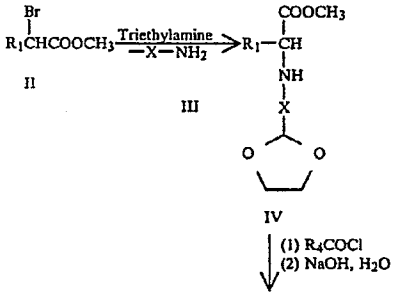
Thus, in compounds of the present invention, the substituent at either position 3 or 4 of the pyrrole nucleus is $-CONR_5R_6$ where R_5 and R_6 are independently hydrogen; alkyl of from one to six carbon atoms; 2-, 3-, or 4-pyridinyl; phenyl; phenyl substituted with fluorine, chlorine, bromine, cyano, trifluoromethyl, or carboalkoxy of from three to eight carbon atoms and the other of the two positions is unsubstituted or is substituted with alkyl of from one to six carbon atoms; cyclopropyl; cyclobutyl; cyclopentyl; cyclohexyl; phenyl; or phenyl substituted with fluorine, chlorine, bromine, hydroxyl; trifluoromethyl; alkyl of from one to four carbon atoms, alkoxy of from one to four carbon atoms, or alkanoyloxy of from two to eight carbon atoms.

Preferred groups for R_5 and R_6 are hydrogen, phenyl, or substituted phenyl. In a particularly preferred group of compounds within the present invention, R_5 is hydrogen and R_6 is phenyl or substituted phenyl.

The compounds of this invention are prepared by the general reaction scheme outlined in Reaction Sequence 1 which takes advantage of the chemistry of mesionic compounds of the type described originally by R. Huisgen et al., *Ang. Chem. Int. Ed.*, 3: 136 (1964).

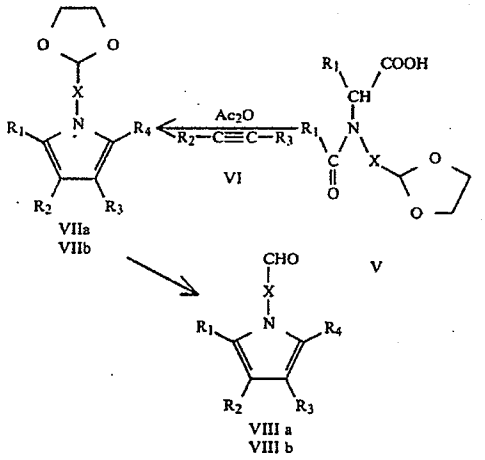
The known, or readily prepared, α-haloesters of structural formula II are reacted with the known 2-[1-(2-aminoalkyl)]-1,3-dioxalane, III, in the presence of an acid scavenger such as triethylamine to produce the N-alkyl-α-aminoesters, IV. The aminoesters, IV are

REACTION SEQUENCE I



-continued

REACTION SEQUENCE I



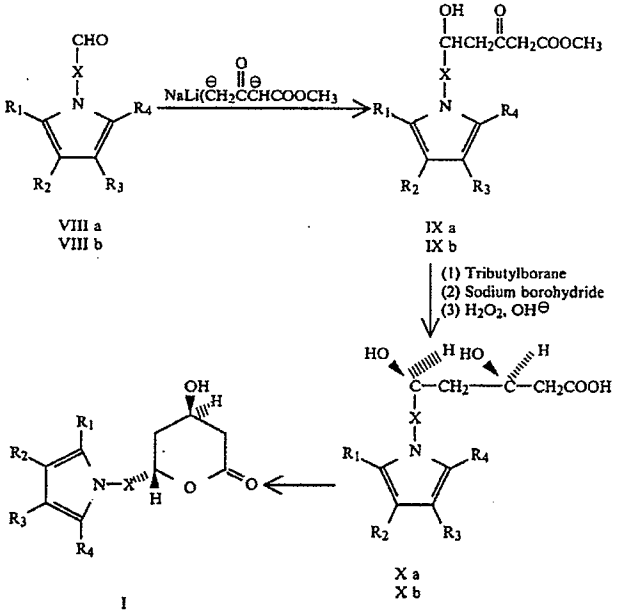
known in the art, and subsequently further purified, if desired, by recrystallization. On the other hand, in the case where R_4 is 1-methylethyl, the cyclo-addition reaction yields predominantly one product which can be purified by recrystallization alone.

Hydrolysis of the acetal function of compounds VIIa and VIIb in aqueous acid solution affords the aldehydes VIIIa and VIIIb. The aldehydes, VIII, are further converted to compounds of the present invention by the processes depicted in Reaction Sequence 2.

The aldehyde compounds, VIII, are reacted with the dilithium or lithio-sodio salt of methyl acetoacetate to produce the corresponding 7-(substituted-pyrrolyl)-5-hydroxy-3-oxoheptanoates, IX. The heptanoates, IX, are dissolved in a polar solvent such as tetrahydrofuran, through which a small amount of air has been bubbled. A slight excess of a trialkylborane, such as tributylborane, is added to the mixture which is then cooled to a temperature of preferably between about 0° C. and −78° C. after which sodium borohydride is added.

The mixture is stirred for about one to two hours and then oxidized by the addition of basic aqueous hydrogen peroxide solution. The reaction produces the 7-(substituted-pyrrolyl)-3,5-dihydroxyheptanoic acids,

REACTION SEQUENCE II



acylated with an acid halide and subsequently hydrolyzed in aqueous base solution to produce the N-acyl-N-alkyl aminoacids, V.

The N-acyl-N-alkyl aminoacids, V, are reacted with the appropriately substituted carboxamido acetylenic compounds, VI, in the presence of an acid anhydride to produce a mixture of the isomeric substituted pyrrole compounds VIIa and VIIb. Depending upon the substituents present, this cyclo-addition reaction affords differing ratios of the two products. For example, in the situation where R_4 is trifluoromethyl, the reaction yields roughly equimolar amounts of the two isomeric products. In such situations, the two isomeric products are separated by chromatographic techniques well

X, in which the product contains a predominance of the desired R*,R* configuration at carbon atoms three and five which bear the hydroxy groups.

The acids may be converted to a corresponding pharmaceutically acceptable salt by conventional means, if desired, or cyclized to the trans-6-[2-(substituted-pyrrol-1-yl)alkyl]pyran-2-ones, I, by dehydration in an inert solvent such as refluxing toluene with azeotropic removal of water. This cyclization step has been found to produce material containing from 85-90% of the desired trans-configuration of the 4-hydroxy group relative to the 6-(substituted-pyrrol-1-yl)alkyl group on the pyran-2-one lactone ring.

The ring-opened hydroxy acids of structural formula II above are intermediates in the synthesis of the lactone compounds of formula I and may be used in their free acid form or in the form of a pharmaceutically acceptable metal or amine salt in the pharmaceutical method of the present invention. These acids react to form pharmaceutically acceptable metal and amine salts. The term "pharmaceutically acceptable metal salt" contemplates salts formed with the sodium, potassium, calcium, magnesium, aluminum, iron, and zinc ions. The term "pharmaceutically acceptable amine salt" contemplates salts with ammonia and organic nitrogenous bases strong enough to form salts with carboxylic acids. Bases useful for the formation of pharmaceutically acceptable nontoxic base addition salts of compounds of the present invention form a class whose limits are readily understood by those skilled in the art.

The free acid form of compounds of the present invention may be regenerated from the salt form, if desired, by contacting the salt with a dilute aqueous solution of an acid such as hydrochloric acid.

The base addition salts may differ from the free acid forms of the compounds of this invention in such physical characteristics as solubility and melting point, but are otherwise considered equivalent to the free acid form for the purposes of this invention.

The compounds of the present invention may exist in solvated or unsolvated form. In general, the solvated forms with pharmaceutically acceptable solvents such as water, ethanol and the like, are equivalent to the unsolvated forms for the purposes of this invention.

The compounds of this invention are useful as hypocholesterolemic or hypolipidemic agents by virtue of their ability to inhibit the biosynthesis of cholesterol through inhibition of the enzyme 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase).

The ability of compounds of the present invention to inhibit the biosynthesis of cholesterol was measured by two methods. A first method (designated CSI screen) utilized the procedure described by R. E. Dugan et al., *Archiv. Biochem. Biophys.*, (1972), 152, 21-27. In this method, the level of HMG-CoA enzyme activity in standard laboratory rats is increased by feeding the rats a chow diet containing 5% cholestyramine for four days, after which the rats are sacrificed.

The rat livers are homogenized, and the incorporation of cholesterol-^{14}C-acetate into nonsaponifiable lipid by the rat liver homogenate is measured. The micromolar concentration of compound required for 50% inhibition of sterol synthesis over a one-hour period is measured, and expressed as an IC_{50} value.

A second method (designated COR screen) employed the procedure detailed by T. Kita, et al., *J. Clin. Invest.*, (1980), 66: 1094–1100. In this method, the amount of ^{14}C-HMG-CoA converted to ^{14}C-mevalonate in the presence of a purified enzyme preparation of HMG-CoA reductase was measured. The micromolar concentration of compound required for 50% inhibition of cholesterol synthesis was measured and recorded as an IC_{50} value.

The activity of several representative examples of compounds in accordance with the present invention appears in Table 1, and is compared with that of the prior art compound, compactin.

For preparing pharmaceutical compositions from the compounds of this invention, inert, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, dispersable granules, capsules, cachets, and suppositories.

A solid carrier can be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, or tablet disintegrating agents; it can also be an encapsulating material.

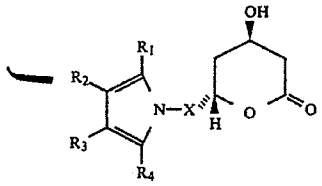
In powders, the carrier is a finely divided solid which is in a mixture with the finely divided active component. In tablets, the active compound is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

For preparing suppositories, a low-melting wax such as a mixture of fatty acid glycerides and cocoa butter is first melted, and the active ingredient is dispersed therein by, for example, stirring. The molten homogeneous mixture is then poured into convenient sized molds and allowed to cool and solidify.

Powders and tablets preferably contain between about 5 to about 70% by weight of the active ingredient. Suitable carriers are magnesium carbonate, magnesium stearate, talc, lactose, sugar, pectin, dextrin, starch, tragacanth, methyl cellulose, sodium carboxymethyl cellulose, a low-melting wax, cocoa butter, and the like.

The term "preparation" is intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component (with or without other carriers) is

TABLE 1



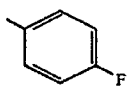
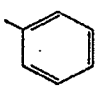
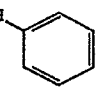
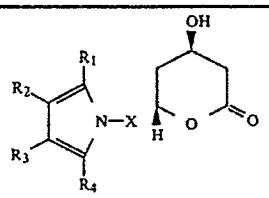
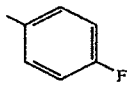
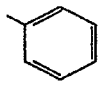
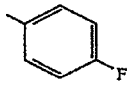
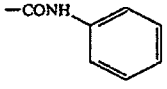
Compound	X	R_1	R_2	R_3	R_4	IC_{50} (Micromoles/liter) CSI	COR
1	$-CH_2CH_2-$			—CONH— 	$-CH(CH_3)_2$	0.035	0.050

TABLE 1-continued



Compound	X	R_1	R_2	R_3	R_4	IC_{50} (Micromoles/liter) CSI	COR
2	$-CH_2CH_2-$		$-CONH-$ 		$-CF_3$	0.40	0.40
3	$-CH_2CH_2-$			$-CONH-$ 	$-CF_3$	0.018	0.020
Compactin (Prior art)						0.026	0.028

surrounded by a carrier, which is thus in association with it. In a similar manner, cachets are also included. Tablets, powders, cachets, and capsules can be used as solid dosage forms suitable for oral administration.

Liquid form preparations include solutions suitable for oral or parenteral administration, or suspensions and emulsions suitable for oral administration. Sterile water solutions of the active component or sterile solutions of the active component in solvents comprising water, ethanol, or propylene glycol may be mentioned as examples of liquid preparations suitable for parenteral administration.

Sterile solutions may be prepared by dissolving the active component in the desired solvent system, and then passing the resulting solution through a membrane filter to sterilize it or, alternatively, by dissolving the sterile compound in a previously sterilized solvent under sterile conditions.

Aqueous solutions for oral administration can be prepared by dissolving the active compound in water and adding suitable flavorants, coloring agents, stabilizers, and thickening agents as desired. Aqueous suspensions for oral use can be made by dispersing the finely divided active component in water together with a viscous material such as natural or synthetic gums, resins, methyl cellulose, sodium carboxymethyl cellulose, and other suspending agents known to the pharmaceutical formulation art.

Preferably, the pharmaceutical preparation is in unit dosage form. In such form, the preparation is divided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of the preparation, for example, packeted tablets, capsules, and powders in vials or ampoules. The unit dosage form can also be a capsule, cachet, or tablet itself, or it can be the appropriate number of any of these packaged forms.

In therapeutic use as hypolipidemic or hypocholesterolemic agents, the compounds utilized in the pharmaceutical method of this invention are administered to the patient at dosage levels of from 40 mg to 600 mg per day. For a normal human adult of approximately 70 kg or body weight, this translates to a dosage of from about 0.5 mg/kg to about 8.0 mg/kg of body weight per day.

The dosages, however, may be varied depending upon the requirements of the patient, the severity of the condition being treated, and the compound being employed. Determination of optimum dosages for a particular situation is within the skill of the art.

The following examples illustrate particular methods for preparing compounds in accordance with this invention. These examples are illustrative and are not to be read as limiting the scope of the invention as it is defined by the appended claims.

EXAMPLE 1

Preparation of trans-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo2H-pyran-2-yl)ethyl]-pyrrole-3-carboxamide

Step A: Preparation of α-[[2-(1,3-dioxalan-2-yl)ethyl]amino]-4-fluorobenzeneacetic acid, ethyl ester

A solution of 26 g (220 mmol) of 2-[1-(2-aminoethyl)]-1,3-dioxalane in 50 ml of acetonitrile was added at room temperature with stirring to a solution of 200 mmol of α-bromo-4-fluorobenzeneacetic acid, ethyl ester (J. W. Epstein et al., *J. Med. Chem.*, 24: 481–490 (1981)) and 42 ml (300 mmol) of triethylamine in 350 ml of acetonitrile. The resulting mixture was stirred at room temperature overnight and then poured into 500 ml of diethyl ether. The resulting suspension was extracted with 300 ml of water and then twice with 300-ml portions of 2M hydrochloric acid. The combined extracts were made basic with 25% aqueous sodium hydroxide solution and extracted twice with 500-ml portions of ethyl acetate. The ethyl acetate extracts were combined, washed successively with water and brine, and then dried over anhydrous magnesium sulfate. The drying agent was removed by filtration, and the residue concentrated to yield 49.5 g of α-[[2-(1,3-dioxalan-2-yl)ethyl]amino]-4-fluorobenzeneacetic acid, ethyl ester.

The 90 MHz proton magnetic resonance spectrum of the product in deuterochloroform exhibited signals at 1.18 (triplet, 3H, J=7 Hz); 1.85 (multiplet, 2H); 2.20

(broad singlet, 1H); 2.6 (multiplet, 2H); 3.85 (multiplet, 4H); 4.1 (quartet, 2H, J=7 Hz); 4.22 (singlet, 1H); 4.83 (triplet, 1H, J=4.5 Hz); and 6.8–7.3 (multiplet, 4H) parts per million downfield from tetramethylsilane.

Step B. Preparation of α-[[2-(1,3-dioxolan-2-yl)ethyl]-(2-methyl-1-oxopropyl)amino]-4-fluorobenzeneacetic acid, ethyl ester.

Thirty grams (100 mmol) of α-[[2-(1,3-dioxalan-2-yl)ethyl]amino]-4-fluorobenzeneacetic acid, ethyl ester from Step A were dissolved in 200 ml of dichloromethane together with 28.6 ml (205 mmol) of triethylamine and the resulting mixture was cooled to 0° C. under dry nitrogen. A solution of 11 ml (105 mmol) of isobutyryl chloride in 50 ml of dichloromethane was slowly added with stirring. After addition was complete, the mixture was stirred for an additional 60 minutes and then poured into 100 ml of diethyl ether. The ether solution was washed successively with portions of water, 2M hydrochloric acid, sodium bicarbonate solution, and brine, and then dried over anhydrous magnesium sulfate. Evaporation of the solvents yielded 35 g of α-[[2-(1,3-dioxolan-2-yl)-ethyl]-(2-methyl-1-oxopropyl)amino]-4-fluorobenzene-acetic acid, ethyl ester.

The 90 MHz proton magnetic resonance spectrum of a deuterochloroform solution of the product exhibited signals at 1.2 (multiplet, 9H); 1.7 (multiplet, 2H); 2.85 (multiplet, 1H); 3.35 (multiplet, 2H); 3.80 (multiplet, 4H); 4.20 (quartet, 2H, J=7 Hz); 4.60 (triplet, 1H, J=4.5 Hz); 5.81 (singlet, 1H); and 6.8–7.3 (multiplet, 4H) parts per million downfield from tetramethylsilane.

Step C. Preparation of α-[[2-(1,3-dioxolan-2-yl)ethyl]-(2-methyl-1-oxopropyl)amino]-4-fluorobenzeneacetic acid

A solution of 35 g (95.3 mmol) of the ester from Step B and 12 g (300 mmol) of sodium hydroxide in 480 ml of 5:1 methanol water was heated under reflux and stirred for two hours. The solution was cooled to room temperature, concentrated, and diluted by the addition of 500 ml of water. The resulting solution was extracted with ether and the aqueous layer was acidified with ice-cold 6M hydrochloric acid and then extracted twice with 300-ml portions of ethyl acetate.

The combined extracts were washed with brine, dried over anhydrous magnesium sulfate, and evaporated to yield 30 g of crude α-[[2-(1,3-dioxolan-2-yl)ethyl]-(2-methyl-1-oxopropyl)amino]-4-fluorobenzeneacetic acid which was used without further purification.

The 90 MHz proton magnetic resonance spectrum of a deuterochloroform solution of the product exhibited signals at 1.11 (doublet, 6H, J=7 Hz); 1.4–1.9 (multiplet, 2H); 2.85 (multiplet, 1H); 3.32 (multiplet, 2H); 3.75 (multiplet, 4H); 4.52 (triplet, 1H, J=4.5 Hz); 5.73 (singlet, 1H); and 6.8–7.3 (multiplet, 4H) parts per million downfield from tetramethylsilane.

Step D. Preparation of N,3-diphenylpropynamide

A solution of 171 mmol of dicyclohexylcarbodiimide in 250 ml of dichloromethane was added dropwise over a two hour period at 0° C. to a suspension of 171 mmol of propiolic acid, 179.6 mmol of aniline, and 5 mmol of 4-dimethylaminopyridine in 400 ml of dichloromethane. After addition was complete, the mixture was stirred for an additional 30 minutes and then diluted with diethyl ether. The resulting mixture was filtered through silica gel, concentrated, and the residue recrystallized to provide 30.5 g of N,3-diphenyl-2-propynamide, mp 122°–123° C.

Analyzed for $C_{15}H_{13}NO$: Calc.: C, 80.69%; H, 5.87%; N, 6.27%; Found: C, 80.54%; H, 5.58%; N, 6.52%.

The infrared spectrum of a KBr pellet of the compound showed principal peaks at 2215, 1630, 1595,1549, 1490, 1445, 1330, 756, and 691 reciprocal centimeters.

Step E. Preparation of 1-[2-(1,3-dioxalan-2-yl)ethyl]-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1H-pyrrole-3-carboxamide

A solution of 95 g (280 mmol) of α-[[2-(1,3-dioxolan-2-yl)ethyl]-(2-methyl-1-oxopropyl)amino]-4-fluorobenzeneacetic acid, prepared as described in Step C above, and 98 g (439 mmol) of N,2-diphenylpropenoic carboxamide, prepared as described in Step D above, was heated at 90° C. with stirring for four hours, (Vigorous gas evolution occurred for two hours.) After this time, the mixture was cooled to room temperature and chromatographed twice on silica gel, eluting with 4:1 hexane:ethyl acetate to separate the product (R_f=0.35) from the starting material (R_f=0.5).

Recrystallization of the product from isopropyl ether provided 59.5 g (119.3 mmol) of 1-[2-(1,3-dioxalan-2-yl)ethyl]-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1H-pyrrole-3-carboxamide, mp 159°–162° C.

Analyzed for $C_{31}H_{31}FN_2O_3$: Calc.: C, 74.68%; H, 6.27%; N, 5.62%; Found: C, 75.04%; H, 6.12%; N, 5.89%.

Step F. Preparation of 5-(4-fluorophenyl)-2-(1-methylethyl)-1-(3-oxopropyl)-N,4-diphenyl-1H-pyrrole-3-carboxamide

A solution of 59 g (118.3 mmol) of 1-[2-(1,3-dioxalan-2-yl)ethyl]-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1H-pyrrole-3-carboxamide, from Step E above, and 0.4 ml of concentrated hydrochloric acid in 1200 ml of anhydrous ethanol was heated under reflux with stirring for 24 hours. After this time the mixture was cooled to room temperature, concentrated, and the residue taken up in 1200 ml of 3:1 acetone:water and 5 g of p-toluenesulfonic acid was added. This mixture was heated under reflux with stirring for two days after which time the solution was cooled to room temperature and partitioned between 1 liter of diethyl ether and 200 ml of brine solution.

The organic phase was separated, washed successively with sodium bicarbonate solution and brine, dried over anhydrous magnesium sulfate and concentrated. The oil which resulted was dissolved in the minimum amount required of hot isopropyl ether. The crystals which formed upon cooling were collected by filtration to yiled 36.8 g of 5-(4-fluorophenyl)-2-(1-methylethyl)-1-(3-oxopropyl)-N,4-diphenyl-1H-pyrrole-3-carboxamide. A further crop of 9.8 g of crystals were obtained from the mother liquor.

Analyzed for $C_{29}H_{27}FN_2O_3$: Calc.: C, 76.63%; H, 5.99%; N, 6.16%; Found: C, 76.48%; H, 6.20%; N, 6.14%.

Step G. Preparation of 2-(4-fluorophenyl)-δ-hydroxy-5-(1-methylethyl)-β-oxo-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, methyl ester

A solution of methyl acetoacetate (26.4 ml, 243 mmol) in 250 ml of anhydrous tetrahydrofuran was added dropwise to a stirred suspension of hexane-washed sodium hydride (6.4 g, 267 mmol) in 200 ml of tetrahydrofuran at 0° C. When gas evolution was complete, 97.2 ml of 2.5M n-butyl lithium was added dropwise over a period of 60 minutes.

The resulting solution was stirred for 30 minutes at 0° C. and then cooled to −78° C. after which a solution of

36.8 g (80.9 mmol) of 5-(4-fluorophenyl)-2-(1-methylethyl)-1-(3-oxopropyl)-N,4-diphenyl-1H-pyrrole-3-carboxamide, from Step F above, in 100 ml of tetrahydrofuran was added over a period of thirty minutes. The resulting solution was stirred for 30 minutes at −78° C. and then warmed to 0° C. where it was held for an additional 60 minutes.

The mixture was then acidified by the dropwise addition of 300 ml of ice-cold 3M hydrochloric acid, diluted with ether, washed successively with water and brine, dried over anhydrous magnesium sulfate, and concentrated. Flash chromatography of the residue yielded 37.9 g of 2-(4-fluorophenyl)-δ-hydroxy-5-(1-methylethyl)-β-oxo-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, methyl ester.

The 90 MHz proton magnetic resonance spectrum of the product exhibited signals at 1.50 (doublet, 6H, J=7 Hz); 1.8 (multiplet, 2H); 2.45 (doublet, 2H, J=7 Hz); 2.8 (broad, 1H); 3.33 (singlet, 2H); 3.5 (multiplet, 1H); 3.67 (singlet, 3H); 3.8–4.0 (multiplet, 2H); and 6.8–7.3 (multiplet, 14H) parts per million downfield from tetramethylsilane.

Step H. Preparation of R*,R*-2-(4-fluorophenyl-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid and trans-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1H-pyrrole-3-carboxamide

Air (60 ml) was bubbled via a syringe through a solution of 2-(4-fluorophenyl)-δ-hydroxy-5-(1-methylethyl)-β-oxo-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, methyl ester (48 g, 84.1 mmol) and 92.5 ml of 1M tributylborane in 100 ml of anhydrous tetrahydrofuran. The mixture was stirred overnight at room temperature and then cooled to −78° C. Sodium borohydride (3.85 g, 101.8 mmol) was added to the cooled mixture in one portion. The mixture was allowed to warm slowly to 0° C. over a period of three hours, during which there was vigorous gas evolution.

The dry ice-acetone bath applied to the reaction vessel was replaced by an ice bath and 18.3 ml of glacial acetic acid were added dropwise, followed by 204 ml of 3M aqueous sodium hydroxide solution and 30.5 ml of 30% aqueous hydrogen peroxide solution.

The mixture was vigorously stirred while being allowed to warm to room temperature overnight. The mixture was then partitioned between diethyl ether and water and the aqueous layer was separated, acidified, and extracted with ethyl acetate.

The ethyl acetate extract was washed with brine, dried, and evaporated to yield crude R*,R*-2-(4-fluorophenyl-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid which was used without further purification.

The crude acid was taken up in toluene and lactonized by heating under reflux for six hours. This mixture was chromatographed to provide 30 g of trans-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1H-pyrrole-3-carboxamide as a foamy solid, mp 90°–97° C.

Analyzed for $C_{33}H_{33}FN_2O_4$: Calc.: C, 73.31%; H, 6.15%; N, 5.18%; Found: C, 73.46%; H, 6.31%; N, 5.28%.

This material was found by HPLC analysis to comprise a 9:1 molar ratio of the cis- and trans-isomeric forms of the product. Recrystallization from toluene-ethyl acetate yield the essentially pure trans-form, mp 148°–149° C.

EXAMPLE 2

Preparation of R*,R*-2-(4-fluoro-phenyl-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, sodium salt

A mixture of trans-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1H-pyrrole-3-carboxamide (10 g, 18.5 mmol) and 0.74 g (18.5 mmol) of sodium hydroxide in 90 ml of a 1:2 mixture of tetrahydrofuran-water was cooled to 0° C. This mixture was allowed to warm slowly to 25° C.; after which time it was concentrated and the residual solid dried under vacuum.

The infrared spectrum of the product exhibited principal absorption peaks at 3400, 1651, 1598, 1565, 1511, 1438, 1412, 1316, 1224, 1159, 844, 754, and 702 reciprocal centimeters.

The 90 MHz proton magnetic resonance spectrum of a hexadeutero dimethylsulfoxide solution of the product exhibited signals at 1.34 (doublet, J=7 Hz, 6H); 1.5 (multiplet, 4H); 1.80 (doublet of doublets, J=15, 8 Hz, 1H); 1.99 (doublet of doublets, J=15, 4 Hz, 1H); 3–4 (multiplet, 8H); 6.9–7.3 (multiplet, 12H); 7.50 (doublet, J=8 Hz, 2H); and 9.85 (singlet, 1H) parts per million downfield from tetramethylsilane.

EXAMPLES 3 AND 4

Preparation of trans-2-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-5-(trifluoromethyl)-pyrrole-3-carboxamide and trans-5-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-2-(trifluoromethyl)pyrrole-3-carboxamide

Step A. Preparation of α-[[2-(1,3-dioxalan-2-yl)ethyl]amino]-4-fluorobenzeneacetic acid.

α-[[2-(1,3-Dioxolan-2-yl)ethyl]amino]-4-fluorobenzeneacetic acid, ethyl ester (36.5 g, 122.8 mmol, prepared as described above in Example 1, Step A) was dissolved in 1500 ml of a 5:1 mixture of methanol-water together with 7.6 g of sodium hydroxide. This mixture was heated under reflux for a period of two and one-half hours after which time the solvents were removed under vacuum.

The solid residue was taken up in 325 ml of water and a mixture of 14 ml of glacial acetic in 28 ml of water was added with stirring. After stirring for a time, an additional 3 ml of glacial acetic acid were added and the mixture was chilled for 75 minutes. The solids were collected by filtration, washed with water and then ethyl acetate and dried to yield α-[[2-(1,3-dioxalan-2-yl)ethyl]amino]-4-fluorobenzeneacetic acid, mp 218°–220° C.

Step B. Preparation of a mixture of 5-(4-fluorophenyl)-1-(3-oxopropyl)-N,4-diphenyl-2-(trifluoromethyl)-1H-pyrrole-3-carboxamide and 2-(4-fluorophenyl)-1-(3-oxopropyl)-N,4-diphenyl-5-(trifluoromethyl)-1H-pyrrole-3-carboxamide

α-[[2-(1,3-Dioxalan-2-yl)ethyl]amino]-4-fluorobenzeneacetic acid (6.06 g, 22.5 mmol) was dissolved in 45 ml of trifluoroacetic anhydride and 7.47 g (33.8 mmol) of N,3-diphenyl-2-propynamide (prepared as described above in Example 1, Step D) was added. The resulting mixture was heated under reflux for a period of five and one-half hours. The mixture was then cooled, and 1.74

ml of trifluoroacetic acid were added and the mixture was stirred overnight.

The excess trifluoroacetic anhydride was removed under vacuum, and water was added, followed by sufficient acetone to give a homogenous solution. This solution was stirred at room temperature for three hours. The mixture was seeded with N,3-diphenyl-2-propynamide, and a precipitate formed. After three hours, this precipitate was removed by filtration.

The acetone was removed from the filtrate under vacuum and the solid residue was taken up in ether, washed successively with two portions of water, two portions of sodium bicarbonate solution, and two portions of brine and dried over anhydrous magnesium sulfate. The ether was removed under vacuum to yield a crude mixture of the two title compounds.

This mixture was separated by column chromatography on 600 g of silica gel, eluting with a 4:1 mixture of hexane-ethyl acetate.

The first fraction eluted was 5-(4-fluorophenyl)-1-(3-oxopropyl)-N,4-diphenyl-2-(trifluoromethyl)-1H-pyrrole-3-carboxamide.

The 90 MHz proton magnetic resonance spectrum of a deuterochloroform solution of this material exhibited signals at 2.73 (triplet, J=7 Hz, 2H); 4.21 (triplet, J=7 Hz, 2H); 6.7–7.3 (multiplet, 5H); 7.40 (singlet, 5H), and 9.43 (singlet, 1H) parts per million downfield from tetramethylsilane.

The second compound eluted from the column was 2-(4-fluorophenyl)-1-(3-oxopropyl)-N,4-diphenyl-5-(trifluoromethyl)-1H-pyrrole-3-carboxamide.

The 90 MHz proton magnetic resonance spectrum of a deuterochloroform solution of this material exhibited signals at 2.67 (triplet, J=7 Hz, 2H); 4.25 (triplet, J=7 Hz, 2H); 7.0–7.3 (multiplet, 14H); and 9.43 (singlet, 1H) parts per million downfield from tetramethylsilane.

Step C. Preparation of trans-2-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-5-(trifluoromethyl)-pyrrole-3-carboxamide and trans-5-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-2-(trifluoromethyl)-pyrrole-3-carboxamide

Employing the general methods detailed in Example 1, Steps G and H, the title compounds were prepared from the aldehyde compounds of this example, Step B.

The elemental analyses of the two title compounds were:

For trans-5-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-2-(trifluoromethyl)-pyrrole-3-carboxamide:

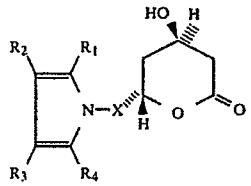
Analyzed for $C_{31}H_{26}N_2O_4$: Calc.: C, 65.72%; H, 4.63%; N, 4.94%; Found: C, 65.82%; H, 4.91%; N, 4.69%.

The trans-2-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-5-(trifluoromethyl)-pyrrole-3-carboxamide was found, upon recrystallization from toluene to contain 0.25 mols of toluene as solvent of crystallization, mp 106°–111° C.

Analyzed for $C_{31}H_{26}N_2O_4.0.25C_7H_8$: Calc.: C, 66.72%; H, 4.79%; N, 4.72%; Found: C, 66.81%; H, 4.86%; N, 4.60%.

I claim:

1. A compound of structural formula I



I

wherein

X is $-CH_2-$, $-CH_2CH_2-$, $-CH_2CH_2CH_2-$, or $-CH_2CH(CH_3)-$;

R_1 is
- 1-naphthyl;
- 2-naphthyl;
- cyclohexyl;
- norbornenyl;
- phenyl;
- phenyl substituted with
  - fluorine,
  - chlorine,
  - bromine,
  - hydroxyl,
  - trifluoromethyl,
  - alkyl of from one to four carbon atoms,
  - alkoxy of from one to four carbon atoms, or
  - alkanoyloxy of from two to eight carbon atoms;

either of R_2 or R_3 is $-CONR_5R_6$ where R_5 and R_6 are independently
- hydrogen;
- alkyl of from one to six carbon atoms;
- phenyl;
- phenyl substituted with
  - fluorine,
  - chlorine,
  - bromine,
  - cyano,
  - trifluoromethyl, or
  - carboalkoxy of from three to eight carbon atoms;

and the other of R_2 or R_3 is
- hydrogen;
- alkyl of from one to six carbon atoms;
- cyclopropyl;
- cyclobutyl;
- cyclopentyl;
- cyclohexyl;
- phenyl; or
- phenyl substituted with
  - fluorine,
  - chlorine,
  - bromine,
  - hydroxyl,
  - trifluoromethyl,
  - alkyl of from one to four carbon atoms,
  - alkoxy of from one to four carbon atoms, or
  - alkanoyloxy of from two to eight carbon atoms;

R_4 is
- alkyl of from one to six carbon atoms;
- cyclopropyl;
- cyclobutyl;
- cyclopentyl;
- cyclohexyl; or
- trifluoromethyl;

or a hydroxy acid or pharmaceutically acceptable salts thereof, corresponding to the opened lactone

ring of the compounds of structural formula I above.

2. A compound as defined by claim 1 wherein X is $-CH_2CH_2-$.

3. A compound as defined by claim 2 wherein R_1 is phenyl; or phenyl substituted with fluorine, chlorine, bromine, hydroxyl; trifluoromethyl; alkyl of from one to four carbon atoms, alkoxy of from one to four carbon atoms, or alkanoyloxy of from two to eight carbon atoms.

4. A compound as defined by claim 2 wherein R_4 is alkyl of from one to six carbon atoms.

5. A compound as defined by claim 1 having the name trans-(±)-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1H-pyrrole-3-carboxamide.

6. A compound as defined by claim 1 having the name trans-2-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-5-trifluoromethyl-1H-pyrrole-3-carboxamide.

7. A compound as defined by claim 1 having the name trans-5-(4-fluorophenyl)-N,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-2-trifluoromethyl-1H-pyrrole-3-carboxamide.

8. A pharmaceutical composition, useful as a hypocholesterolemic agent, comprising a hypocholesterolemic effective amount of a compound in accordance with claim 1 in combination with a pharmaceutically acceptable carrier.

9. A method of inhibiting cholesterol biosynthesis in a patient in need of such treatment by administering a pharmaceutical composition as defined by claim 8.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE EXTENDING PATENT TERM UNDER 35 U.S.C. § 156

PATENT NO.	:	4,681,893
ISSUED	:	July 21, 1987
INVENTOR(S)	:	Bruce D. Roth
PATENT OWNER	:	Warner-Lambert Company

This is to certify that there has been presented to the

COMMISSIONER OF PATENTS AND TRADEMARKS

an application under 35 U.S.C. § 156 for an extension of the patent term. Since it appears that the requirements of the law have been met, this certificate extends the term of the patent for the period of

1,213 days

from May 30, 2006, the original expiration date of the patent, subject to the provisions of 35 U.S.C. § 41(b), with all rights pertaining thereto as provided by 35 U.S.C. § 156(b).

I have caused the seal of the Patent and Trademark Office to be affixed this 15th day of July 1998.

Bruce A. Lehman

Bruce A. Lehman
Assistant Secretary of Commerce and
Commissioner of Patents and Trademarks

Berg Report Exhibit E


US006569459B2

(12) **United States Patent**
Flashner-Barak

(10) **Patent No.: US 6,569,459 B2**
(45) **Date of Patent: May 27, 2003**

(54) **METHOD OF ADMINISTRATION OF PACLITAXEL-PLASMA PROTEIN FORMULATION**

(75) Inventor: **Moshe Flashner-Barak**, Petach Tikva (IL)

(73) Assignee: **Teva Pharmaceutical Industries, Ltd.**, Petah Tiqva (IL)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/829,744**

(22) Filed: **Apr. 10, 2001**

(65) **Prior Publication Data**

US 2001/0056070 A1 Dec. 27, 2001

Related U.S. Application Data

(60) Provisional application No. 60/195,912, filed on Apr. 10, 2000.

(51) **Int. Cl.**[7] **A61K 9/14**; A61K 9/50; A61F 2/00

(52) **U.S. Cl.** **424/489**; 424/484; 424/486; 424/499; 424/400; 424/426

(58) **Field of Search** 424/489, 484, 424/401, 426, 486, 499; 514/449

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,407,683	A	4/1995	Shively	
5,415,869	A	5/1995	Straubinger et al.	
5,470,311	A	11/1995	Setterstrom et al.	
5,496,846	A	3/1996	Wilson et al.	
5,504,102	A	4/1996	Agharkar et al.	
5,580,575	A	12/1996	Unger et al.	
5,621,001	A	4/1997	Canetta et al.	
5,626,862	A	5/1997	Brem et al.	
5,641,803	A	6/1997	Carretta et al.	
5,651,986	A	7/1997	Brem et al.	
5,684,169	A	11/1997	Hamada et al.	
5,696,153	A	* 12/1997	Ainsworth et al.	514/449
5,846,565	A	12/1998	Brem et al.	
5,888,530	A	3/1999	Netti et al.	
6,071,952	A	* 6/2000	Owens et al.	514/449

FOREIGN PATENT DOCUMENTS

WO	WO 99/13914	* 3/1999

OTHER PUBLICATIONS

Kuh HJ, Jang SH, Wientjes MG, Weaver JR, Au JL–S. Determinants of paclitaxel penetration and accumulation in human solid tumor. Journal of Pharmacol Exp Ther. Aug. 1999; 290:871–880.*

Gisele Sarosy and Eddie Reed, Journal of the National Medical Association, Jun. 1993, vol. 85, No. 6, pp. 427 to 431.

* cited by examiner

Primary Examiner—Thurman K. Page
Assistant Examiner—Rachel M. Bennett
(74) *Attorney, Agent, or Firm*—Kenyon & Kenyon

(57) **ABSTRACT**

The present invention provides for a method for treating human or animal patients with paclitaxel formulation, the method comprising an intratumoral dose of a paclitaxel formulation and an intravenous dose of paclitaxel. The intravenous dose occurs about 1 to about 7 days after the intratumoral dose. The paclitaxel formulation may typically be either a paclitaxel/HSA formulation or paclitaxel/γ-globulin formulation.

20 Claims, No Drawings

METHOD OF ADMINISTRATION OF PACLITAXEL-PLASMA PROTEIN FORMULATION

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of provisional application Ser. No. 60/195,912, filed Apr. 10, 2000, which is incorporated entirely herein by reference.

FIELD OF THE INVENTION

The present invention relates to the field of delivery of anti-tumor chemotherapeutics and more particularly to delivery of the anti-tumor chemotherapeutic, paclitaxel.

BACKGROUND

Paclitaxel is a high molecular weight (854 g/mole), highly lipophilic cytotoxic chemotherapeutic used as an anti-tumor agent in the treatment of carcinomas of the ovary, breast, lung and in the treatment AIDS related Karposi's sarcoma. Paclitaxel is currently used to treat breast cancer by pre-operatively administering the drug systemically. The pre-operation treatment reduces tumor burden prior to surgery, thus potentially improving the post-surgery prognosis. Although impressive success has been achieved using this approach, the treatment requires prolonged hospitalization and is accompanied by severe side-effects. Moreover, a significant number of cases (30%) do not result in a clinically satisfactory outcome either because the tumors are not reduced or because the side effects require that paclitaxel dosing be discontinued.

Paclitaxel's cytotoxic and anti-tumor properties derive from its ability to promote apoptosis (programed cell death) by inducing the assembly of microtubules from tubulin dimers and preventing microtubules from depolymerization. The stabilized microtubules inhibit normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic functions. In addition paclitaxel induces abnormal arrays or "bundles" of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis.

Paclitaxel Formulations

Paclitaxel is substantially water insoluble and must be administered using a solubilizing carrier. The currently approved paclitaxel carrier formulation, marketed as TAXOL®, comprising pacitaxel dissolved in ethanol and CREMOPHOR®EL (polyoxyethylated castor oil).

The TAXOL® carrier CREMOPHOR®EL can cause side effects, such as anaphylaxis and severe hyper-sensitivity. (Sarosy and Reed, *J Natl Med Assoc* (1993) 85(6):427–31.) To reduce the side effects, current recommended treatment with TAXOL® includes pre-medication with corticosteroids, diphenhydramine and H_2 antagonists.

Several alternative carriers have been proposed to address the anaphylaxis and severe hyper-sensitivity caused by the CREMOPHOR®EL. For example, U.S. Pat. No. 5,684,169, which is incorporated by reference, discloses unbranched cyclodextrin or branched cyclodextrin inclusion complexes of paclitaxel which improves the solubility of paclitaxel in water. The complex is produced by adding an unbranched cyclodextrin or a branched cyclodextrin to paclitaxel at a molar ratio of 1–20 times with respect to paclitaxel. By improving solubility, the cyclodextrin inclusion complex improves paclitaxel absorption in cancer patients.

U.S. Pat. No. 5,415,869, which is incorporated by reference, discloses paclitaxel or paclitaxel tumor-active

analogs solubilized using one or more negatively charged phospholipids and one or more zwitterionic phospholipids. The phospholipid mixture entraps paclitaxel or the analog in a liposome. The liposome is in the form of particles having a size of 0.025 to 10 microns, with substantially no crystals of paclitaxel or the analog.

U.S. Pat. No. 5,580,575, which is incorporated by reference, discloses a therapeutic drug delivery system comprising gas-filled microspheres and a therapeutic drug, as well as, methods for employing such microspheres in therapeutic drug delivery. The preferred microspheres of the disclosure are gas-filled liposomes with an encapsulated drug. Methods of preparing such liposomes in drug delivery applications are also disclosed.

WO 99/13914, incorporated herein by reference, discloses that paclitaxel, and other slightly water soluble drugs can be formulated without CREMOPHOR®EL or other toxic solubilizers by forming a water soluble homogeneous complex with plasma proteins, such as human serum albumin (HSA) or human gamma globulin (γ-globulin). As disclosed by WO 99/13914 homogeneous aqueous solutions up to at least 4.68 mM paclitaxel (4 mg/mL) can be formulated using HSA. The plasma proteins act as a slow release depot of paclitaxel. WO 99/13914 further discloses a dosage range of paclitaxel-HSA complex containing 70–280 mg of paclitaxel per treatment. Such formulations can be made bio-equivalent to the conventional CREMOPHOR®EL containing formulations.

Other formulations for administering paclitaxel are disclosed in U.S. Pat. Nos. 5,504,102 and 5,407,683, incorporated herein by reference.

In addition, the slow infusion of CREMOPHOR®EL solutions has been studied as a means of avoiding or ameliorating the side effects of the CREMOPHOR®EL vehicle. The most common dosage is 135–175 mg/m^2 CREMOPHOR®EL, which is administered over a 3 hour, 6 hour, or 24 hour dosage schedule. (See U.S. Pat. Nos. 5,641,803, and 5,621,001, both incorporated herein by reference.) Other dosing schedules have been suggested to reduce toxic side effects, including 96 hour infusion every 21 days (U.S. Pat. No. 5,496,846, incorporated herein by reference) and 60–180 minutes, repeated a plurality of times during a 21 day period, each infusion separated by an interval of between 4 to 5 days. (U.S. Pat. No. 5,696,153, incorporated herein by reference).

Paclitaxel Chemotherapy Reservoir

An alternative method of administering paclitaxel is using a chemotherapy reservoir. U.S. Pat. Nos. 5,846,565, 5,626,862 and 5,651,986, which are incorporated by reference, discloses a method and devices for localized delivery of a chemotherapeutic agent to solid tumors, where the chemotherapeutic agent does not cross the blood-brain barrier and is characterized by poor bioavailability and/or short half-lives in vivo. The devices consist of reservoirs which release the chemotherapeutic over an extended period while at the same time preserving the bio-activity and bio-availability of the agent. The preferred embodiment is biodegradable polymeric matrices. Alternatively reservoirs can be made from non-biodegradable polymers or reservoirs connected to implanted infusion pumps. The devices are implanted within or immediately adjacent to the tumors to be treated or the site where tumors have been surgically removed. The patents further disclose the efficacy of paclitaxel and camptothecin delivered in polymeric implants prepared by compression molding of biodegradable and non-biodegradable polymers, respectively.

U.S. Pat. No. 5,888,530, which is incorporated by reference, discloses a method of enhancing the amount of a pharmaceutical composition delivered to a target tissue site in a mammal, by creating a transient differential between the hydrostatic pressure in the target site and a region near the target tissue site. An apparatus for performing the method is provided. In one form that apparatus includes a pharmaceutical reservoir, pump, and an agent reservoir and pump.

Chemotherapy reservoirs are also disclosed in U.S. Pat. No. 5,470,311 incorporated herein by reference.

Initial results testing such chemotherapy reservoirs have been disappointing. While a significantly lowered side effect profile has been demonstrated, there are no indications of clinical improvement.

The limitations of current chemotherapy reservoir technology is probably due to the retention of the chemotherapeutic drug only on the tumor periphery or at the injection site due to the poor penetration and distribution of the drug as a result of the neoplasm's high interstitial fluid pressure. A more potent anti-tumor effect can be achieved by targeting the chemotherapy directly to the tumor, i.e., intratumorally, rather than by systemic infusion.

We now report a method of delivering an anti-cancer chemotherapeutic, such as paclitaxel, by first administered paclitaxel by intratumoral injection and thereafter administering paclitaxel by intravenous injection. This invention takes advantage of the lower toxicity and side effects of paclitaxel/plasma solutions, and the ability of plasma proteins, such as HSA, to act as a slow release depot for paclitaxel.

SUMMARY OF THE INVENTION

The present invention provides for a method of delivering paclitaxel, the method comprising an intratumoral dose of a paclitaxel formulation and an intravenous infusion of paclitaxel wherein the intravenous infusion occurs about 24 hours to about 7 days after the intratumoral dose.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for a method of delivering a paclitaxel. According to the invention paclitaxel, as a paclitaxel formulation, is first brought into contact with substantially all the cells of a solid tumor, by an intratumoral dose. Thereafter paclitaxel is administered by intravenous infusion. The paclitaxel administered by intravenous infusion may be the same paclitaxel formulation used in the intratumoral dose. Alternatively, the paclitaxel may be administered by infusion of paclitaxel in any other soluble form.

While not being bound by theory, it is believed that the intratumoral dose of the paclitaxel formulation induces apoptosis within the tumor by slowly releasing paclitaxel into the tumor over a period of twenty-four hours to one week. The cell death that occurs within the tumor results in the collapses of the tumor structure. The collapsed tumor allows access of the second intravenous dose of paclitaxel to reach inside the partially collapsed tumor structure. One of skill in the art will recognize that, the invention is not limited to methods which function in this manner.

Intratumoral Dose of Paclitaxel Formulation

One aspect of the present invention provides for introducing a paclitaxel formulation intratumorally. For example, in one embodiment of the present invention, the intratumoral dose of paclitaxel formulation may be injected intratumorally using a syringe pump. The flow rate and pressure of the syringe pump will depend upon the tumor to be treated. The flow rate of the syringe pump may vary from about 0.0167 ml/min to about 0.5 ml/min. The preferred flow rate will deliver the paclitaxel formulation to greater than 90% of the tumor volume while delivering essentially no paclitaxel outside the tumor.

The paclitaxel formulation is preferably a soluble form of paclitaxel comprising a paclitaxel/plasma protein complex. As used herein, paclitaxel/plasma protein complex refers to paclitaxel in a water-ethanol solution containing a solubilizing amount of plasma protein wherein the paclitaxel forms a non-covalent complex with the plasma protein. Preferably the plasma protein is HSA or γ-globulin. Most preferably the plasma protein is HSA. One of skill in the art will understand that paclitaxel/plasma protein is not limited to the use of these two proteins and includes any plasma protein capable of forming a non-covalent paclitaxel/plasma protein complex and thereby solubilizing paclitaxel.

While not being bound by theory, it is proposed that administering a soluble form of paclitaxel, such as a paclitaxel/plasma protein complex, increases drug efficacy by promoting paclitaxel diffusion. Increased diffusion promotes apoptosis tumor cell death not only in the immediate zone of the injection but also at sites further into the tumor where the paclitaxel has migrated.

The mass of paclitaxel formulation delivered intratumorally depends upon the size of the tumor, and can range up to about 280 mg of paclitaxel. Preferably, the intratumoral mass of paclitaxel is from about 1 to about 60 mg of paclitaxel.

The volume of the dose is preferably about ¼ to about 1/12 the tumor volume. Most preferably the volume of the dose is about 1/10 of the tumor volume.

The preferred concentration of the paclitaxel formulation is about 4 to about 10 mg/ml of paclitaxel, or about 3.4 to about 8.5 mM paclitaxel.

Thus, a tumor with a 4 cm diameter has a volume of 33 cc. Consequently, a 6 ml of a 10 mg/ml dose of paclitaxel liquid delivered into the tumor results in a dose of 60 mg paclitaxel which is approximately the maximal intratumoral injection dose.

If the initial intratumoral administration of the paclitaxel formulation does not substantially shrink the solid tumor, an additional intratumoral dose of the paclitaxel formulation may be administered. The additional intratumoral dose may be at an identical, at a greater, or at a lower concentration of the paclitaxel formulation then the initial intratumoral dose. In one embodiment of the present invention, the paclitaxel/plasma protein may be administered by multiple intratumoral injections within a short period of time. The invention provides for multiple intratumoral injections of the paclitaxel formulation administered within 24 hours.

While not being bound by theory, administering the paclitaxel formulation by frequent intratumoral injections may increase the efficacy of paclitaxel by inducing apoptosis at various stages of the cell cycle.

Intravenous Infusion of Paclitaxel

Methods of delivering an anti-tumor chemotherapeutic by intravenous infusion are well known in the art and are described for example in U.S. Pat. Nos. 5,696,153, 5,496,846, and 5,641,803.

In one embodiment of the present invention, the paclitaxel is administered by intravenous infusion about twenty-four hours to about 1 week following the intratumoral dose as a

continuous infusion. The intravenous dose is typically administered over about 3 to about 12 hours. The paclitaxel administered by intravenous infusion may be the paclitaxel formulation used in the intratumoral dose administered as a saline solution of 5% dextrose or normal saline. Alternatively, the paclitaxel may be administered by infusion of paclitaxel in any other soluble form.

When the paclitaxel administered by intravenous infusion comprises the paclitaxel/plasma protein complex, the intratumoral or the intravenous treatment may be repeated in cycles. The administration of the paclitaxel/plasma protein complex may be repeated because of the decreased hypersensitivity reaction from the paclitaxel paclitaxel/plasma protein complex compared to TAXOL®.

In one embodiment, the intravenous infusion of paclitaxel comprises administering a plurality of repeated intravenous infusions subsequent to the intratumoral dose, wherein each infusion is separated by about seven days.

Another embodiment comprises administering an additional intravenous infusion of the paclitaxel formulation, about 4 to about 21 days subsequent to the intravenous infusion.

Another embodiment comprises an additional intratumoral dose administered subsequent to the intravenous dose. The additional intratumoral dose is preferably administered about 4 to about 21 days subsequent to the intravenous infusion.

In an alternative embodiment of the invention, the intravenous dose may be administered by solubilizing paclitaxel in CREMOPHOR®EL ethanol solutions. The solution of this embodiment comprises 6 mg/mL paclitaxel, corresponding to a paclitaxel concentration of about 7 mM, which is diluted prior to infusion with 0.9% sodium chloride injection, U.S.P, 5% dextrose injection, U.S.P, 5% dextrose and 0.9% sodium chloride injection, U.S.P, or 5% dextrose in Ringer's injection to a final concentration of 0.3 to 1.2 mg/mL. The maximum TAXOL® concentration which can be administered by intravenous infusion using this formulation is about 0.6 mg/mL.

The intravenous dose is preferably in the range of about 100 to about 200 mg/m^2. More preferably the intravenous dose is in the range of about 135 to about 175 mg/m^2.

The mass of paclitaxel administered by intravenous infusion is preferably between about about 70 to about 280 mg.

The function and advantage of these and other embodiments of the present invention will be more fully understood from the examples below. The following examples are intended to illustrate the benefits of the present invention, but do not exemplify the full scope of the invention.

EXAMPLES

EXAMPLE 1

Improved Spread of Evan's Blue-Albumin in a Human Mammary Adenocarcinoma MCF7 Xenograft in Immunocompromised Mice when Injecting Intratumorally Under Pressure as a Model for Paclitaxel/HSA Spread in the Tumor.

Purpose

The purpose of the study is to assess the efficiency of spread of a solution of Evan's blue dye—albumin complex in a tumor when injected intratumorally at different flow rates. The complex of the dye with albumin serves as a model of the complex of paclitaxel with albumin and allows visualization of the complex spread within the tumor.

Methods and Results

Nude (athymic mice) (~5 weeks of age) were injected subcutaneously with a cell suspension containing approximately 10^7 cells/0.1 ml of human mammary tumor cell line MCF7. On Day 28 following tumor cell implantation, all tumors were measured as described below, and the measurement recorded for each mouse as the pre-treatment baseline tumor volume. Tumor measurement were performed using calipers, to measure the tumor in two dimensions, at approximately 90°. to each other, at the longest and widest points. The tumor volume was calculated according to the formula, $(W^2 \times L)/2$, where W is the tumor measurement at the widest point, and L is the tumor dimension at the longest point.

Mice with tumor volumes within the range of 5–8 grams were allocated to the study. The mice were injected intratumorally with 1 ml of a solution of Evan's blue albumin in buffered saline using a Sage Instrument Model #355 syringe pump. The albumin dye complex serves as a visual model for the albumin paclitaxel complex. The solution was injected into the tumor at various flow rates between 0.0167 ml/min to 0.5 ml/min which corresponded to various back pressures. The faster the flow rate the higher the (not measured) back pressure is presumed to be. The flow rates tested were:

Flowrate
0.0167 ml/min (1 ml/60 min)
0.05 ml/min (1 ml/20 min)
0.1 ml/min (1 ml/10 min)
0.2 ml/min (1 ml/5 min)
0.5 ml/min (1 ml/2 min)

After the injections the mice were sacrificed, the tumor removed and the extent of the spread of the blue dye in the tumor measured visually.

From the results are given in the following table. One can see that the raising of the pressure results in a more efficient spread of the dye.

Flow rate	Percent of tumor volume dyed
0.0167 ml/min (1 ml/60 min)	2–5
0.05 ml/min (1 ml/20 min)	20–40
0.1 ml/min (1 ml/10 min)	40–60
0.2 ml/min (1 ml/5 min)	70–90
0.5 ml/min (1 ml/2 min)	>90

Conclusion

The results exemplify that the albumin can be effectively spread within the entire tumor volume when the pressure of the infusion is slightly raised. In our system, a flow rate of 0.2 ml/min suffices to raise the pressure and spread the soluble albumin complex. The efficient spread of the paclitaxel albumin complex results in more efficacious treatment of the solid tumors.

EXAMPLE 2

In Vivo Evaluation of the Anti-Tumor Effect of Intratumoral Injections of Paclitaxel/HSA in Human Breast Tumor (Cell line MCF7) Xenografts in Nude Mice, Purpose of Study

The purpose of the study is to assess the anti-tumor effect of intratumoral injections of Paclitaxel/HSA, a novel pro-

prietary compound of paclitaxel complexed with human serum albumin) against a human mammary tumor xenograft (cell line MCF7) in immunodeficient mice. The potential of paclitaxel/HSA to reduce tumor size is compared to the standard chemotherapeutic agent, TAXOL®.

Methods and Results

There are five study groups containing 6–10 mice per group. The mice are allocated to the following 5 groups:

Group Number	Drug	Dosage	Method of Administration	Number of Injections (within 24 hours)
I	No treatment (control)	—	—	—
II	Saline (control)	0.2.ml/gm[a]	Intratumoral	2
III	TAXOL ®	0.2 ml/gm[a]	Intratumoral	2
IV	Paclitaxel/HSA	0.2 ml/gm[a]	Intratumoral	2
V	Paclitaxel/HSA	0.2 ml/gm[a]	Intratumoral (via high-pressure infusion)	2

[a]per gram tumor weight at 1 mg paclitaxel/ml

Nude (athymic mice) (~5 weeks of age) are injected subcutaneously with a cell suspension containing approximately 10^7 cells/0.1 ml of human mammary tumor cell line MCF7. The mice are examined routinely for the appearance of tumors. On Day 28 following tumor cell implantation, all tumors are measured as described below, and the measurement recorded for each mouse as the pre-treatment baseline tumor volume. Tumor measurement are performed using calipers, to measure the tumor in two dimensions, at approximately 90° to each other, at the longest and widest points. The tumor volume are calculated according to the formula, $(W^2 \times L)/2$, where W is the tumor measurement at the widest point, and L is the tumor dimension at the longest point.

All mice with tumor volumes within the range of 5–8 grams are allocated to study groups. Allocation to treatment groups are carried out based on the volume of the individual tumors, with each study group receiving an approximately equal representation of all tumor volumes. At study baseline, Day "0" of the Treatment Phase, all mice are receive the first injection according to their study group assignment. Approximately twenty-three hours later, the tumors are measured as described above, and the volumes recorded. Immediately following measurement, within 24 hours of the first injection, the mice are receive a second injection according to the study group assignment. Post-treatment tumor volumes are assessed at 48 hours, 7 days, 14 days, and 21 days following the initial injection. The mice are sacrificed and the tumors removed and weighed. The final weights for each treatment group are averaged and compared to the final weights obtained for the "no-treatment" group.

For each mouse within a study group, the post-treatment tumor volumes just before the 2^{nd} injection at 24 hours, and at 48 hours, 7, 14 and 21 days following the initial injection, are measured and recorded. The relative tumor volume (post-treatment tumor volume/pre-treatment baseline tumor volume) are recorded at each time point, and the mean relative tumor volume for each time point, for all mice within a study group, are determined. Additionally, following sacrifice, the final weights for the tumors for each study group are averaged and compared to the final weights observed for the "no-treatment" group.

The expected results of the measurement of relative tumor volume (100×post-treatment tumor volume/pre-treatment baseline tumor volume) (expected results) are tabulated in the following table:

Group	% tumor volume at 2 days	% tumor volume at 7 days	% tumor volume at 14 days	% tumor volume at 21 days
I	105	125	150	175
II	105	125	150	170
III	50	50	75	85
IV	40	40	60	75
V	40	25	25	40

Conclusion

Intratumoral injections of soluble paclitaxel/HSA are an effective method of affording tumor shrinkage. Two intratumoral injections separated by 24 hours are effective in shrinking the tumor to about 40% of its original value. Elevated pressure makes the injections more effective. Further injections, in an improved protocol, could conceivably bring about a full remission in the tumor.

What is claimed is:

1. A method of administering paclitaxel to a patient having a tumor, the method comprising;

introducing an intratumoral dose of a paclitaxel formulation; and,

subsequently providing an initial intravenous infusion of paclitaxel about 24 hours to about 7 days after the intratumoral dose.

2. The method of claim **1**, wherein the paclitaxel formulation is a mixture of paclitaxel and plasma protein in an amount effective to solubilize the paclitaxel.

3. A method of treating a patient having a tumor comprising:

administering to a tumor at least one intratumoral dose of a first formulation comprising paclitaxel and a plasma protein, thereby inducing apoptosis; and

administering intravenously to a patient at least one dose of a second formulation comprising paclitaxel, the first dose of the second formulation occurring about 1 to about 7 days after the the intratumoral dose,

wherein the first and second formulations are the same or different, and

wherein the amount of plasma protein in the first formulation is an amount effective to solubilize the paclitaxel.

4. The method of claim **3**, wherein the plasma protein is selected from the group consisting of human serum albumin and γ-globulin.

5. The method of claim **3**, wherein the dose of paclitaxel formulation is between about 1 to about 60 mg of paclitaxel.

6. The method of claim **3**, wherein the paclitaxel formulation is between about 4 to about 10 mg/ml paclitaxel.

7. The method of claim **3**, wherein the intratumoral dose is administered by a plurality of injections of the paclitaxel formulation.

8. The method of claim **3**, wherein the intratumoral dose of the paclitaxel formulation is administered by syringe pump.

9. The method of claim **3**, wherein the intravenous infusion of paclitaxel comprises administering between about 70 to about 280 mg of paclitaxel.

10. The method of claim **3**, wherein the intravenous infusion of paclitaxel comprises administering between about 100 to about 200 mg/m^2 of paclitaxel.

11. The method of claim **3**, wherein the intravenous infusion of paclitaxel comprises administering between about 135 to about 175 mg/m^2 of paclitaxel.

12. The method of claim **3**, wherein the intravenous infusion of paclitaxel comprises administering a mixture of

paclitaxel and plasma protein in an amount effective to solubilize the paclitaxel.

13. The method of claim 12, wherein the solubilizing plasma protein is selected from the group consisting of human serum albumin and γ-globulin.

14. The method of claim 1, wherein the intravenous infusion of paclitaxel comprises administering paclitaxel and polyoxyethylated castor oil.

15. The method of claim 3, wherein the intravenous infusion of paclitaxel comprises administering a plurality of intravenous infusions subsequent to the intratumoral dose.

16. The method of claim 3, further comprising administering an additional intravenous infusion of the paclitaxel formulation subsequent to the intravenous infusion.

17. The method of claim 16, wherein the additional intravenous dose is administered about 4 to about 21 days subsequent to the intravenous infusion.

18. The method of claim 16, further comprising administering an intratumoral dose of the paclitaxel formulation subsequent to the additional intravenous infusion.

19. The method of claim 3, further comprising administering an additional intratumoral dose of the paclitaxel formulation subsequent to the intravenous infusion.

20. The method of claim 19, wherein the additional intratumoral dose is administered about 4 to about 21 days subsequent to the intravenous infusion.

* * * * *

EXHIBIT B

Westlaw.
Not Reported in F.Supp.
Not Reported in F.Supp., 1997 WL 158281 (D.Del.)
(Cite as: Not Reported in F.Supp.)

H

Briefs and Other Related Documents

Revlon Consumer Products Corp. v. L'Oreal S.A.D.Del.,1997.Only the Westlaw citation is currently available.

United States District Court,D. Delaware.

REVLON CONSUMER PRODUCTS CORPORATION, Plaintiff,

v.

L'ORÉAL S.A., Cosmair, Inc., Maybelline, Inc., and Maybelline Sales, Inc., Defendants.

Civil Action No. 96-192 MMS.

March 26, 1997.

Jack Blumenfeld, Jon E. Abramczyk, Morris, Nichols, Arsht & Tunnell, Wilmington, DE, Daniel J. Leffell, Elizabeth J. Holland, Douglas A. Berman, Paul, Weiss, Rifkind, Wharton & Garrison, New York City, John W. Behringer, Fitzpatrick, Cella, Harper & Scinto, of Counsel, for plaintiff.

Rudolph E. Hutz, Stanley C. Macel, III, Connolly, Bove, Lodge & Hutz, Wilmington, DE, Norman H. Stepno, Frederick G. Michaud, Jr., David M. Schlitz, Ronni S. Jillions, Burns, Doane, Swecker & Mathis, L.L.P., Alexandria, VA, Norman F. Oblon, Richard D. Kelly, Jean-Paul Lavalleye, Frank J. West, Oblon, Spivak, McClelland, Maier & Neustadt, P.C., Arlington, VA, of Counsel, for defendants.

MEMORANDUM OPINION

MURRAY M. SCHWARTZ, Senior District Judge.

INTRODUCTION

***1** Revlon Consumer Products Corp. ("Revlon") filed this lawsuit against L'Oréal S.A., Cosmair Inc., Cosmair Canada, Inc.,[FN1] Maybelline, Inc. and Maybelline Sales, Inc. (collectively "defendants") alleging infringement of Revlon's patented composition for transfer resistant lipstick. *See* Docket Item ("D.I.") 61 (Amended Complaint). Three defendants, Cosmair Inc., Maybelline Inc., and Maybelline Sales Inc., asserted a counterclaim seeking a declaratory judgment that Revlon's patent is invalid and they have not infringed nor induced infringement.

FN1. Cosmair Canada, Inc. has since been dismissed as a defendant. D.I. 24.

Before the Court is Revlon's motion to preclude the testimony of defendants' patent law expert, John Witherspoon. D.I. 147. According to his report, Mr. Witherspoon proposes to offer opinions on a wide range of issues, including Patent and Trademark Office ("PTO") practice and procedure as well as many substantive areas of patent law.[FN2] *Id.,* Exh. A at 2. The parties agree Mr. Witherspoon may testify as to PTO practice and procedure. D.I. 154, at 1; D.I. 157 at 2. Revlon asserts, however, the remainder of Mr. Witherspoon's proposed testimony goes to topics inappropriate for expert testimony in a patent case. D.I. 157, at 2. In their answer to Revlon's motion, defendants indicate other than PTO practice and procedure, they wish only to introduce Mr. Witherspoon's testimony on the issue of inequitable conduct. D.I. 154, at 1. Thus, to resolve Revlon's motion, the Court must decide whether to admit testimony by a proffered patent law expert on the topic of inequitable conduct.

FN2. Specifically, these areas are: "patent infringement, both literal and under the doctrine of equivalents; principles of claim construction and interpretation; prosecution history estoppel; conditions for patentability, including novelty, utility and nonobviousness under 35 U.S.C. § § 101, 102 and 103; requirements for and purposes of patent specifications and claims under 35 U.S.C. § 112; the prohibition regarding the addition of new matter under 35 U.S.C. § 132; duties and responsibilities of an inventor, his or her attorney or agent, and others substantively involved in the preparation and prosecution of a patent application in the PTO; and the prosecution history of the patent in suit." D.I. 147, Exh. A, at 2.

DISCUSSION

I. Inequitable Conduct

Inequitable conduct has been defined by the Federal Circuit Court of Appeals as "an 'affirmative misrepresentation of a material fact, failure to

disclose material information, or submission of false material information, coupled with an intent to deceive.' " *Micro Chemical, Inc. v. Great Plains Chemical Co., Inc.,* 103 F.3d 1538, 1549 (Fed.Cir.1997) (citation omitted); *accord Refac International, Ltd. v. Lotus Development Corp.,* 81 F.3d 1576 (Fed.Cir.1996). "Information is 'material' when there is a substantial likelihood that a reasonable examiner would have considered the information important in deciding whether to allow the application to issue as a patent." *Refac International,* 81 F.3d at 1581.

The proponent of a claim of inequitable conduct must prove "the threshold elements of materiality and intent by clear and convincing evidence." *Micro Chemical, Inc.,* 103 F.3d at 1549. "The district court must then weigh the threshold findings of materiality and intent in light of all the circumstances to determine whether they warrant a conclusion that inequitable conduct occurred." *Id.* "A determination of inequitable conduct is committed to a district court's discretion." *Id.*

II. Expert Testimony

Defendants assert Mr. Witherspoon's testimony as to inequitable conduct may assist the trier of fact and thus is admissible under Federal Rule of Evidence 702. That rule states:

***2** If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise.

Fed.R.Evid. 702.

Because the admission of expert testimony is a "procedural matter" not unique to patent issues, the law of the Third Circuit Court of Appeals governs this motion, as opposed to the law of the Federal Circuit. *Panduit Corp. v. All States Plastic Manufacturing Co.,* 744 F.2d 1564, 1574-75 (Fed.Cir.1984); *accord National Presto Industries, Inc. v. The West Bend Co.,* 76 F.3d 1185, 1188 n. 2 (Fed.Cir.1996).

The decision whether to admit expert testimony is committed to the discretion of the district court. *United States v. Velasquez,* 64 F.3d 844, 847-48 (3d Cir.1995). As might be gleaned from the rule, several bases exist for excluding expert testimony. They are: "(1) if the testimony will not assist the trier of fact; (2) if scientific [or technical or other specialized] evidence is not sufficiently reliable; and (3) if the particular expert does not have sufficient specialized knowledge to assist the jurors." *Petruzzi's IGA Supermarkets, Inc. v. Darling-Delaware Co.,* 998 F.2d 1224, 1238 (3d Cir.1993); *see also Holbrook v. Lykes Bros. Steamship Co., Inc.,* 80 F.3d 777, 781 (3d Cir.1996).

The Third Circuit Court of Appeals has adopted a broad interpretation of Rule 702; close calls on the admission of expert testimony are to be resolved in favor of admissibility. *Dunn v. Hovic,* 1 F.3d 1362, 1367 (3d Cir.1993). However, "it is not permissible for a witness to testify as to the governing law since it is the district court's duty to explain the law to the jury...." *United States v. Leo,* 941 F.2d 181, 196 (3d Cir.1991). As relevant to Revlon's motion, Mr. Witherspoon's testimony will be inadmissible either if it is not helpful to the trier of fact, or if it constitutes impermissible testimony before the jury as to the governing law.

Defendants have not provided the details of Mr. Witherspoon's proposed testimony on inequitable conduct, beyond the sentence: "Defendants request that Mr. Witherspoon be allowed to testify as to the inequitable conduct issue if the Court determines that Mr. Witherspoon's testimony as a legal expert would assist in its determination." D.I. 154, at 2. Defendants' answer to Revlon's motion places into issue the currently unsettled question of whether, in this case, the judge or the jury will act as fact-finder on the issue of inequitable conduct.

With respect to that question, the Federal Circuit recently explained:

There are a variety of ways in which the district court may choose to handle the issue of inequitable conduct during a jury trial. .... Some courts have reserved the entire issue of inequitable conduct unto themselves; some have submitted special interrogatories to the jury on the facts of materiality and intent; and some have instructed the jury to find and weigh the facts of materiality and intent and decide the ultimate question of inequitable conduct.... Absent a clear showing of prejudice, or failure to achieve a fair trial, the district court's choice of procedure will not be disturbed.

***3** *Hebert v. Lisle Corp.,* 99 F.3d 1109, 1114 (Fed.Cir.1996). The court noted in the last instance the parties agreed to submit the entire issue of inequitable conduct to the jury. *Id.*

Failing to achieve similar agreement of the parties in the present case, the Court will opt to submit to the jury special interrogatories on the facts of materiality and intent. The Court will then weigh the findings on these two elements "in light of all the circumstances," and decide the ultimate question of inequitable conduct. *See Micro Chemical, Inc.,* 103 F.3d at 1549; *see also Akzo N.V. v. U.S. International Trade Commission,* 808 F.2d 1471, 1481-82 (Fed.Cir.1986) ("Materiality and intent must ... be considered together: the more material the omission or misrepresentation, the less intent that must be shown to reach a conclusion of inequitable conduct.")

As the determination of the Court consists of a 'weighing' of the factual findings on materiality and intent, and then a determination in light of all the circumstances whether inequitable conduct occurred, *see Micro Chemical. Inc.,* 103 F.3d at 1549, it follows that the jury will act as the sole fact-finder on the issue of inequitable conduct. The Court therefore cannot permit Mr. Witherspoon to testify as an expert on inequitable conduct; to do otherwise would usurp the respective functions of the jury and the Court. [FN3]

> FN3. The Federal Circuit recently noted one of the hazards of permitting expert testimony on patent law:
> We take note of the extent to which ... incorrect law was announced by a patent law expert witness. We encourage exercise of the trial court's gatekeeper authority when parties proffer, through purported experts ... markedly incorrect law.
> *Hebert,* 99 F.3d at 1117.

In accordance with the other cases in this District, the Court holds defendants' expert John Witherspoon may testify only as to matters of PTO practice and procedure. *See Lucas Aerospace, Ltd. v. Unison Industries, L.P.,* No. 93-525 (D.Del. March, 9, 1995); *General Battery Corp. v. Gould, Inc.,* 545 F.Supp. 731, 758 n. 30 (D.Del.1982); *see also Thorn EMI North America Inc. v. Micron Technology, Inc.* No. 92-673 (D.Del. Nov.23, 1993) (McKelvie, J.) (hearing transcript); *The Read Corporation v. Portec, Inc.,* No. 88-29 (D.Del. March 9, 1990) (Roth, J.) (hearing transcript); *RCA Corp. v. Data General Corp.,* No. 84-270 (D.Del. Dec. 17, 1986) (Farnan, J.) (hearing transcript); Guidelines: Legal Expert Testimony in Patent Cases (Robinson, J.).[FN4]

Mr. Witherspoon may not testify as to substantive issues of patent law, including inequitable conduct. For purposes of clarity, it is noted this holding precludes, among other things, Mr. Witherspoon's proposed testimony regarding the "duties and responsibilities of an inventor, his or her attorney or agent, and others substantively involved in the preparation and prosecution of a patent application in the PTO...." D.I. 147, Exh. A, at 2.

> FN4. While this rule regarding patent experts is followed in this District, it is not uniform throughout the country. Several Federal Circuit cases refer, in passing, to expert testimony that was permitted on the topic of inequitable conduct, *see Hebert,* 99 F.3d at 1115; *Kingsdown Medical Consultants Ltd. v. Hollister, Inc.,* 863 F.2d 867, 872 (Fed.Cir.1988).

An order will issue consistent with this opinion.

D.Del.,1997.
Revlon Consumer Products Corp. v. L'Oreal S.A.
Not Reported in F.Supp., 1997 WL 158281 (D.Del.)

Briefs and Other Related Documents (Back to top)

• 1:96cv00192 (Docket) (Apr. 12, 1996)

END OF DOCUMENT